REMARKS

Claims 47, 52 53, 67, and 79-80 have been canceled, claims 44, 48-51, 54-61, 64-66, 68-70, and 73-78 have been amended, and claims 81-86 have been added. The second "claim 76" presented in the amendment filed May 27, 2007, is now claim 86.

An office action was mailed October 10, 2007, and applicant filed a response on March 10, 2008. On June 25, 2008, an office action issued in which the Examiner stated that the March 10, 2008 response was deemed non-responsive because it presented claims to an invention distinct from that previously examined. Accordingly, the claim amendments presented in the March 10 response were not entered. The claim amendments presented above are based on the claims as pending prior to the March 10 response. Applicant below addresses the rejections set forth in the October 10, 2007 Office Action.

In the October 10, 2007, Office Action, claims 44, 47-61, 64-65, 67 and 71-80 were rejected under 35 USC § 103(a) as obvious over Geng in view of Nelson. The specific grounds for rejection, and applicant's response thereto, are set forth in detail below.

Support for claim amendments

The amended claims are fully supported by the specification and original claims as set forth in detail below:

Claim 44: support for the *contacting* element can be found at page 19, lines 21-23; support for the *anti-peptide* element can be found at page 17, line 20 *et seq*; support for the *specific for said first peptide* element can be found at page 18, lines 6-7; support for the *known quantity* element can be found at page 19, lines 12-16; support for the eluting element can be found at page 12, lines 15-22 and page 19, lines 24-26; support for the measuring *using a mass spectrometer* element can be found at page 21, line 23 *et seq*; and support for the *proteolytic digest* element can be found at page 8, lines 15-18 and page 18, lines 20-23.

Claims 48-51: support for the *monoclonal antibody* and *polyconal antibody* elements can be found at page 8, lines 21-22.

Claims 54 and 65: support for the *substituted for the corresponding predominant natural isotope in more than 98%* element can be found at page 17, line 12-14.

Claims 55-59: support for the *antibody* element can be found at page 12, lines 15-17, page 37, lines 31-34, page 28, lines 13-18, original claim 16, and page 29, lines 1-3, respectively.

Claim 60: support for the *set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer* element can be found at page 15, line 9 *et seq*.

Claim 61: see generally, support for the amendment to claim 44.

Claim 64: support for the deletion can be found at page 17, line 3.

Claims 73 and 76: support for the *at least 100 fold* element can be found at page 38, lines 2-3.

Claims 74 and 77: support for the *anti-peptide* element can be found at page 17, line 20 *et seq*.

Claims 78 and 81: support for the *subjected to a concentrating step after elution from said antibodies and before introduction into said mass spectrometer* element can be found at page 30, lines 6-14.

Claims 82-83: support for the news claims can be found at page 8, lines 21-22 and page 11, lines 13-29.

Claims 84-85: support for the new claims can be found at page 22, lines 6-10.

Claim 86: support for the new claim can be found in previously filed duplicate claim 76.

Rejection under 3 USC § 103(a)

Claims 44, 47-61, 64-65, 67 and 71-80 are rejected under 35 USC § 103(a) as obvious over Geng in view of Nelson. Specifically, the Examiner states that Geng describes sample digestion, affinity separation of peptide fragments, and mass spectrometric ("MS") analysis of the separated fragments. The Examiner further states that Geng describes isotopically-labeled peptides and use of those peptides as standards in MS analysis of the peptide fragments obtained by affinity purification. The Examiner admits that Geng fails to teach use of antibodies as binding agents, but states that this deficiency is remedied by Nelson. The Examiner states that Nelson describes use of affinity capture using antibodies to overcome signal suppression and matrix saturation effects in MS analysis of complex biofluids and to increase sensitivity. Based on these alleged disclosures in the prior art, the Examiner states that it would have been obvious

to modify the analytical method described by Geng by using antibodies as described by Nelson because Nelson describes the use of antibodies to capture antigen for MS analysis. In addition, the Examiner states that one of ordinary skill in the art would have had a reasonable expectation of success in using antibodies because only "routine skill" was required to use antibodies to capture antigen and that "the expected results would have been obtained." Finally, the Examiner asserts that the instantly claimed methods amount to "routine optimization" by making "changes in amounts of an old process" and, as such, is not patentable. Applicants respectfully traverse.

The present invention provides new and powerful methods for studying protein expression in complex mixtures, such as those found in bodily fluids. The claimed methods are quantitative and are capable of studying specific individual proteins that are present in low or high abundance. The specificity and sensitivity of the claimed methods are highly surprising and represent a significant advance over prior art methods, as described in more detail in the Declaration by Dr. Steven Carr ("the Carr Declaration") appended hereto as EXHIBIT 1.

The Carr Declaration describes how prior methods were not only unable to provide specificity by unambiguously identifying single molecules from complex mixtures, but also failed to address the problems associated with studying proteins that are present in low abundance in a complex mixture that contains many proteins present in high abundance. Carr Declaration at paragraphs 8-14. MS methods are most effectively used in studying shorter peptides, which can be obtained by protease digests of intact proteins. Carr Declaration at paragraphs 4-5. However, proteolysis of complex protein mixtures, such as bodily fluids, generates such a large number of peptides that study of the peptides by prior art methods was an intractable problem. Carr Declaration at paragraph 11. Moreover, this complexity was made more difficult by the widely varying abundance of proteins present in bodily fluids. Carr Declaration at paragraph 12. Dr. Carr, an expert in the field, and others in the field, were surprised that the claimed methods worked at all to enable study of proteins present at low abundance in complex mixtures such as bodily fluids. Carr Declaration at paragraphs 15-16. The ability to study low abundance proteins is important, for example, when one is studying molecules that are biomarkers or candidate biomarkers, as these typically are present in low abundance. Carr Declaration at paragraphs 4-5.

By contrast, the methods described in Geng and Nelson do not teach or suggest the instantly claimed methods, and provide no hint or suggestion that the instantly claimed methods should be tried at all, let alone that the claimed methods would provide high sensitivity and high specificity. Accordingly, the cited references fail to present a *prima facie* case of obviousness and the rejection should be withdrawn.

Geng fails to describe methods of general application

As applicant has previously explained, the methods described by Geng are designed to study a class of proteins (glycoproteins) and not individual proteins. Thus, Geng describes use of a lectin as an affinity reagent that binds and isolates glycopeptides *as a class* of molecule. As described in Figure 1 of Geng, a tryptic digest is passed down a lectin column, thereby separating glycopeptides from non-glycosylated peptides. The resulting glycopeptides can then be analyzed as a class. The methods described by Geng therefore are limited to glycopeptides and, *ipso facto* are unsuitable for study of non-glycosylated peptides. Moreover, not only do the Geng methods lack specificity, but they fail to provide any solution to the problem of studying low abundance proteins in the presence of large numbers of high abundance proteins. Indeed, the deficiencies and lack of general applicability are candidly acknowledged in Geng itself at the paragraph bridging pages 305 and 306, which states:

> Limitations of the method are ****that only the particular type of post-translational modification selected can then be identified. **It remains to be determined whether affinity selection will be of utility in cases where different post-translational modifications, such as phosphorylation and acylation, reside within the same peptide.**

(Emphasis supplied). This is an explicit admission *by the authors* that the methods described by Geng were of very limited applicability and were not predictive of results using other affinity-based selection approaches. Moreover, it is notable that Geng provides not a single mention of antibody-based approaches, which is powerful evidence that the instantly claimed methods would not have been obvious to one of ordinary skill in the art at the time the instant application was filed. If use of antibodies for affinity purification and MS analysis of peptides from complex mixtures of peptides present in bodily fluids was so obvious, why does Geng fail to make even a single mention of antibody methods? The answer is, of course, that such methods never had

occurred to Geng. Moreover, even if Geng somehow had taught or suggested antibody-based methods (which it did not), the strong cautionary language quoted above regarding the unpredictability surrounding the general application of Geng's methods could not possibly suggest to one of ordinary skill in the art that there was a reasonable expectation of success that antibody methods would be successful. For at least these reasons, applicant respectfully submits that no *prima facie* case of obviousness exists and requests withdrawal of the rejection.

Nelson describes only isolation of intact protein and fails to teach or suggest methods of isolating peptides from complex mixtures

The Examiner states that Nelson describes methods of affinity capture using antibodies to overcome signal suppression and matrix saturation effects in MS analysis of complex biofluids and to increase sensitivity. Nelson is, however, solely directed to a method of isolating a single intact protein from a mixture and studying that protein. Nelson fails completely to teach or suggest proteolytic digestion of a complex protein mixture such as a bodily fluid to produce peptides, nor how one could then isolate and study particular peptides in that complex mixture, let alone study low abundance peptides in the presence of large numbers of high abundance peptides. If it was so obvious to use MS to study peptides in complex digests, why does Nelson fail to teach or suggest proteolytic digestion to produce such peptides? The answer, of course, is that Nelson's object is to enrich and characterize intact proteins present in a biological sample, and therefore digestion of the proteins to produce peptides would be counterintuitive, to say the least. Moreover, by solely concerning itself with intact proteins, rather than peptides, Nelson not only fails to solve, but fails completely to recognize the problems associated with analysis of complex mixtures of peptides produced from low and high abundance proteins discussed in detail above and in the Carr Declaration. Accordingly, Nelson cannot provide a reasonable expectation of success in using antibody-based separation methods to study peptides produced from complex mixtures such as bodily fluids, let alone address the problems identified by Geng and discussed in detail above. For at least these reasons, applicant respectfully submits that no *prima facie* case of obviousness exists and requests withdrawal of the rejection.

There was no reasonable expectation of success

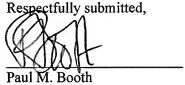
The Examiner states that one of ordinary skill in the art would have had a reasonable expectation of success in using antibodies to isolate peptides for MS analysis because only "routine skill" was required to use antibodies to capture antigen and that "the expected results would have been obtained." Applicants respectfully traverse.

For the reasons explained in the Carr Declaration, not only was there no reasonable expectation of success in using the instantly claimed methods, but experts in the field were very surprised that the methods worked at all. This is powerful evidence of non-obviousness and applicant respectfully requests withdrawal of the rejection.

CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully submit that the application is in condition for allowance. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact the undersigned to expedite prosecution of the application.

Respectfully submitted,



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